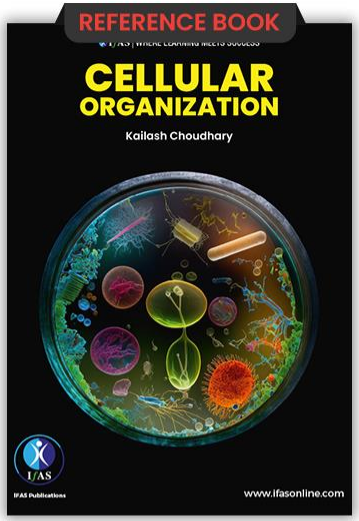
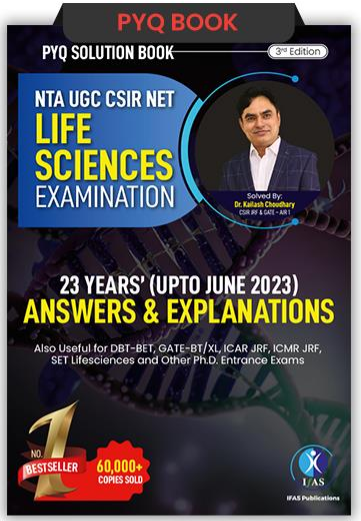
Don't wait for the opportunity. Create it.

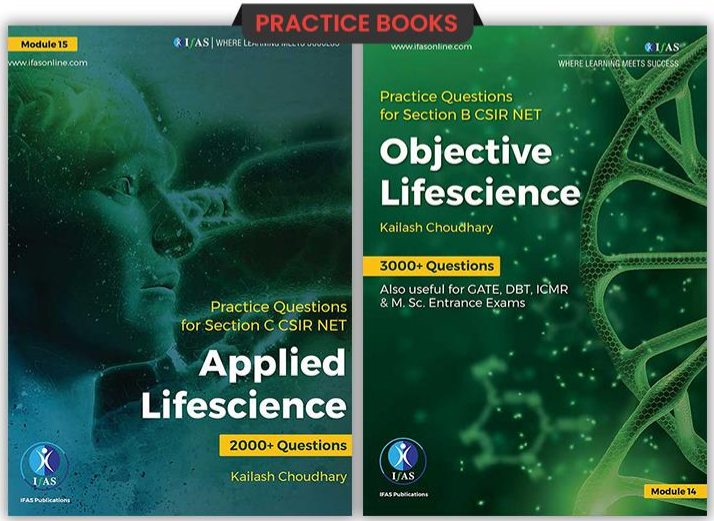
CSIR NET – Life Science

Unit 2: Cellular Organization

L12 Transposons and Cytoskeleton







IfAS

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Points to be covered in this Lecture

- **Transposons**
- Cytoskeleton
- Microfilaments: Structure, associated proteins, function, drugs
- Microtubule

Transposon.

Trans = change,
Poson = position

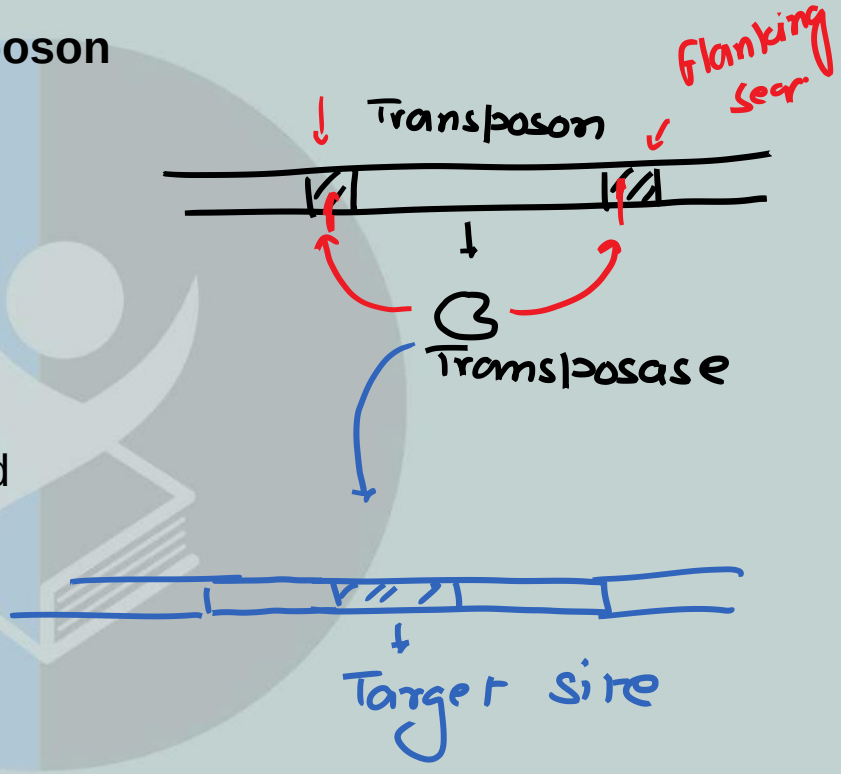
Transposon is the DNA element that can change their position within chromosome or between chromosome.

It is known as mobile DNA elements or jumping gene.

General characteristics of Transposon

1. Contains transposase enzyme.

- Helps in transposition.
- Recognize flanking sequence of Transposon.
- Induce cut-on flanking sequence and
- transfer it into new target site.



Transposon.

DNA transposon

- most common in prokaryotic organism.
- Also present in lower eukaryotes.

Retrotransposon

It is most common in higher eukaryotic organism.

DNA transposon.

Non-replicative

-> Cut-paste type

-> most common among prokaryote

examples.

(1) Insertion element i.e. IS-1, IS-2, IS-3 etc.

(2) Composite transposon i.e. TN-5, TN-10, TN-9 etc.

(3) C elegans transposon.

(4) P element of Drosophila.

(5) AC-DS element.

Replicative

- Copy-paste type.
- Rare type
  - TN-3.
  - Mu-phage

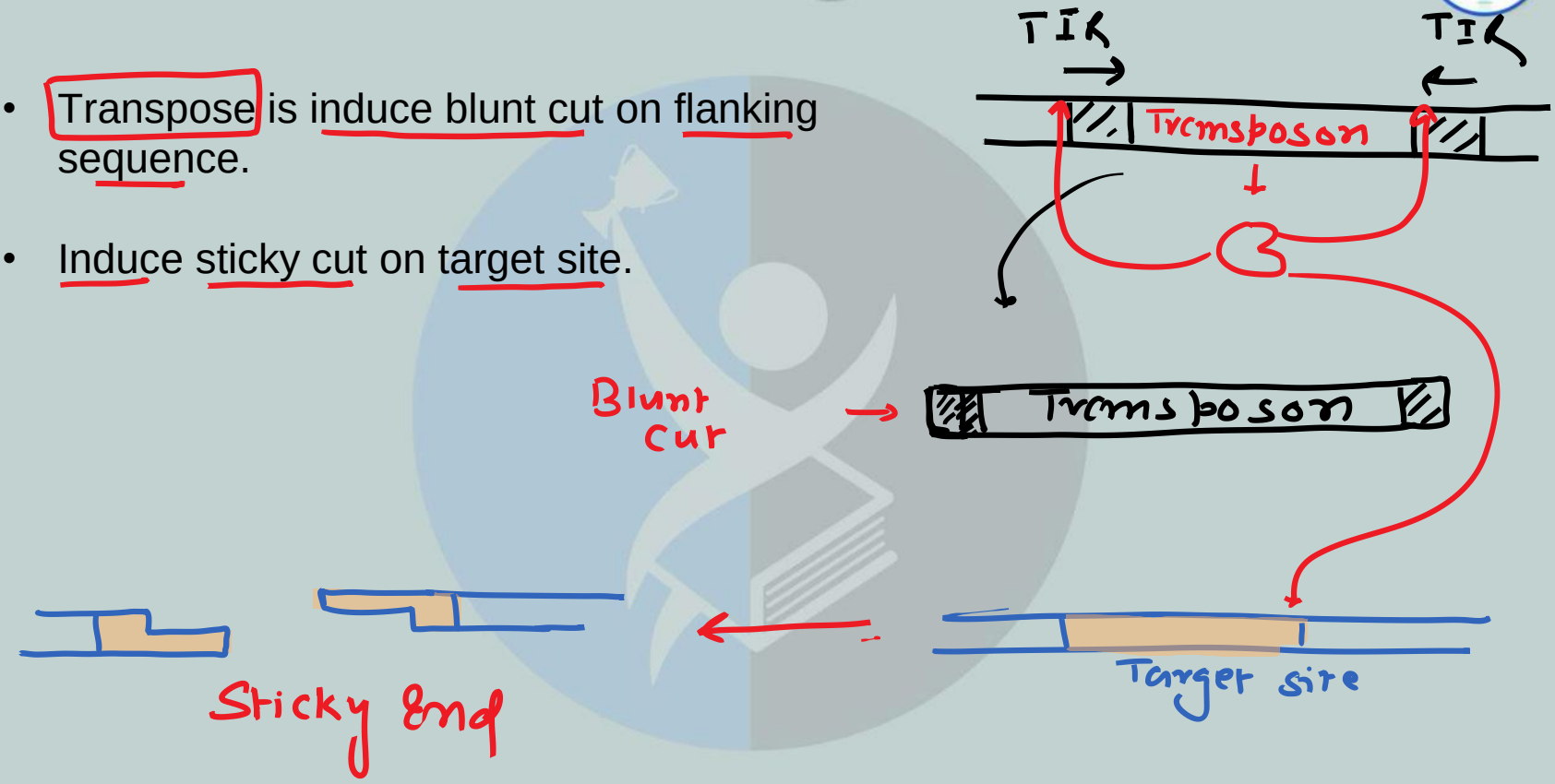
non-replicative transposon. [Cut · Paste · type]

1. Contain terminal inverted repeats. [Flanking Seq]

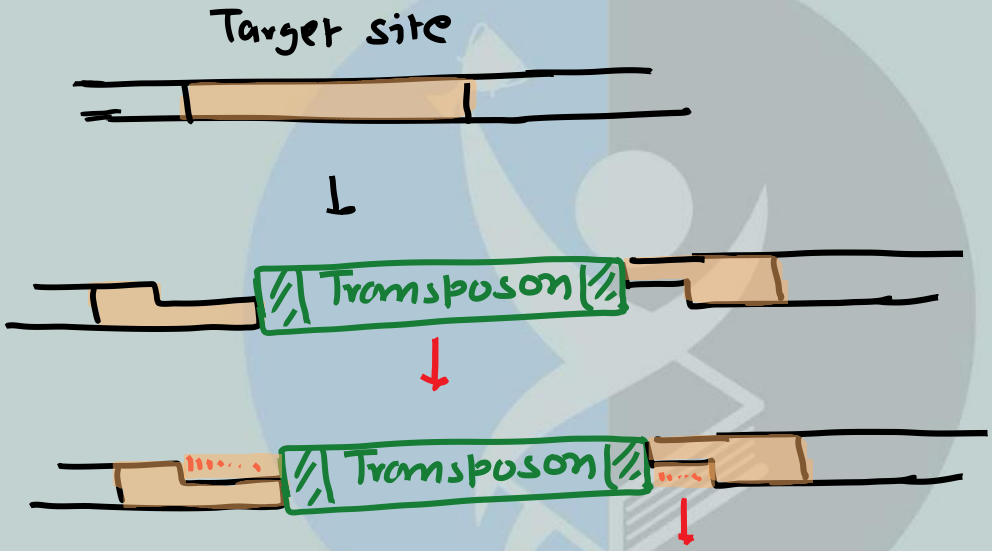
2. Transpose is present.

. Recognize TIR.
. Induce cut on TIR.
. Excise DNA from old site and paste into another new target site.

- Transpose is induce blunt cut on flanking sequence.
- Induce sticky cut on target site.



3. Target site duplication.



4. Transposon can be recognized by

- TIR.
- Duplicated target site.

Examples –

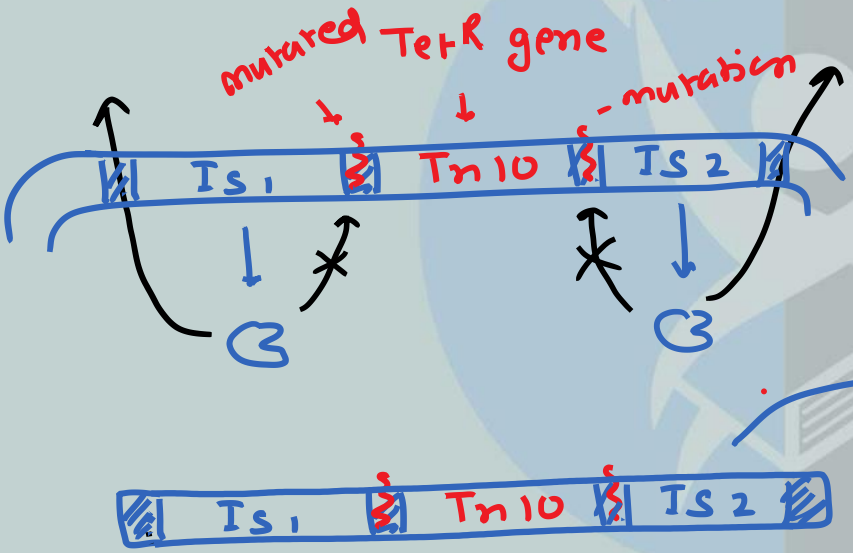
1. **Insertion element.** → most common Among Prokaryote
   - Size 700-2500 base pairs.
   - TIR present.
   - Transpose gene present.
   - Target site duplication.

Discovered by Jacob & monod

IS-1, IS-2, IS-3 etc.

2. Composite transposon.

2 insertion elements are Tn



Transposition of whole seq.

↳ Tn 10 is sandwich b/w IS1 & IS2

Examples

- TN-10 is equal to Tetracycline resistant gene.
- TN-9 is equal to Chloramphenicol resistant gene.
- TN-5 kanamycin resistant gene.

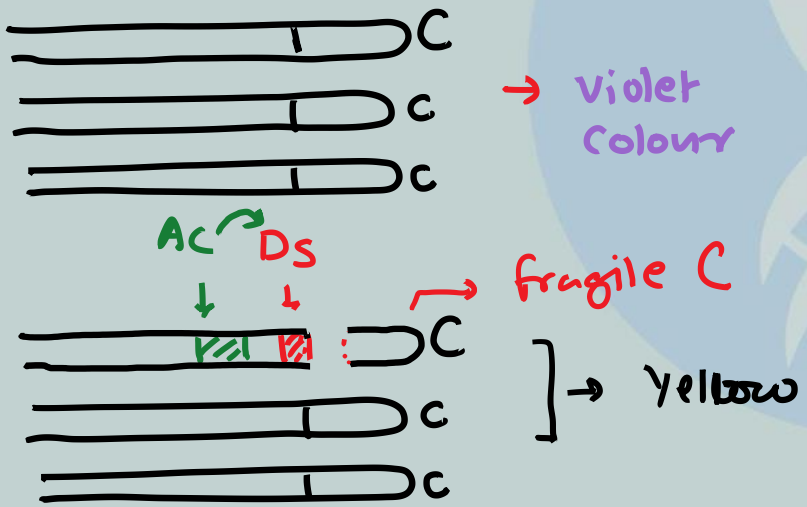
3. AC-DS element. [in maize by Barbara Macklintock]

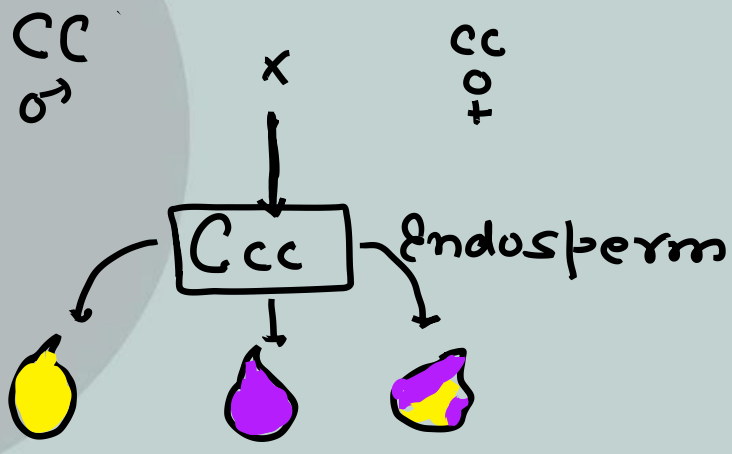
AC = activation

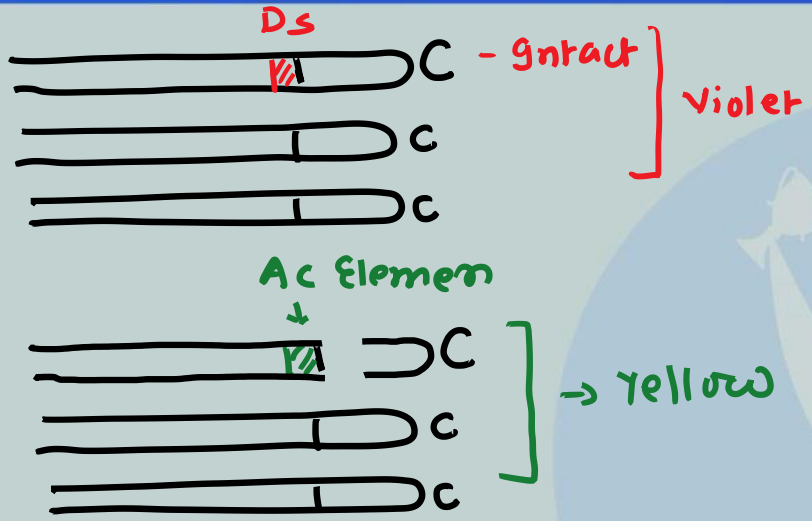
DS = dissociation element.

- Gene that is responsible for endosperm color is present on chromosome number 9.
- CC represent violet color dominant.
- cc yellow recessive.

Triplet





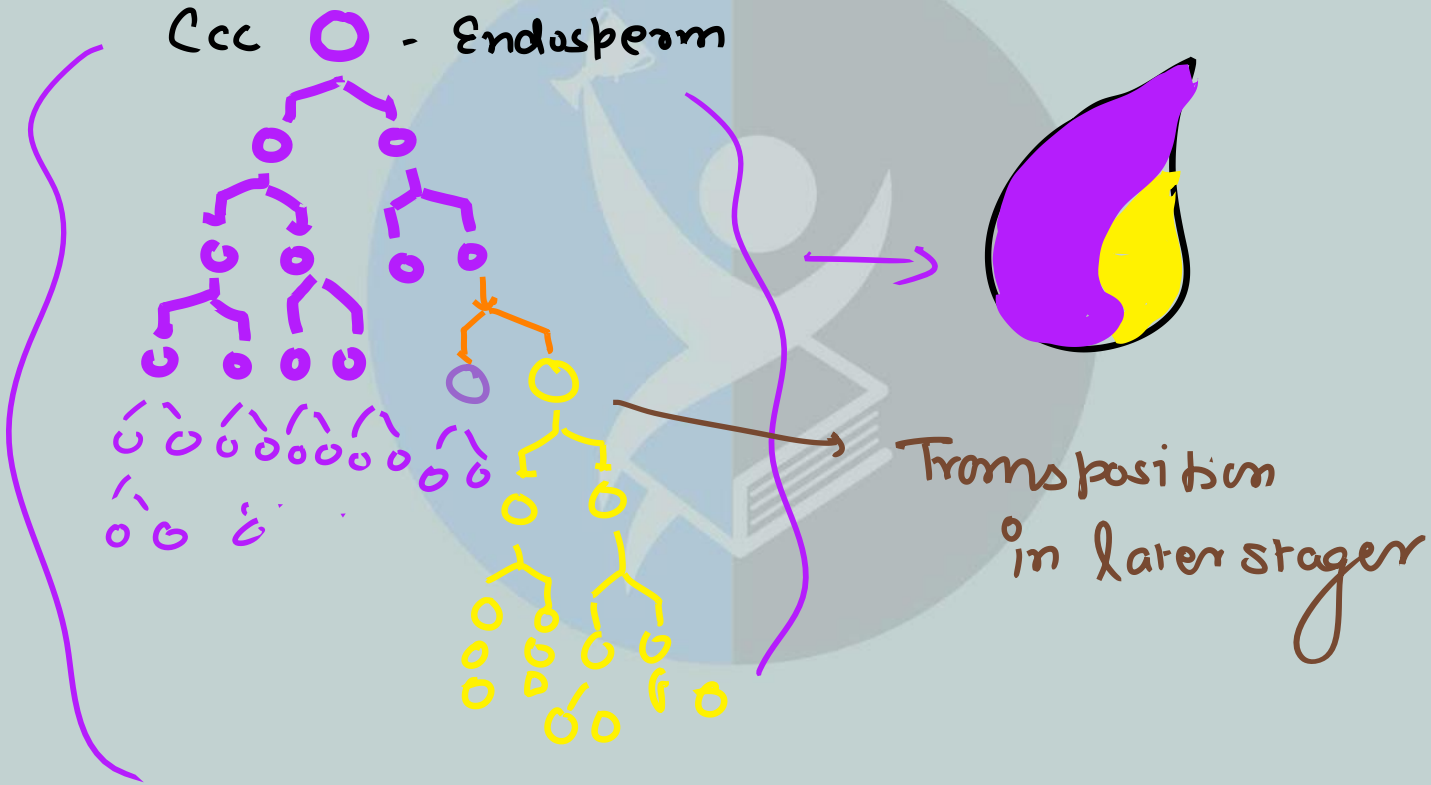


- AC element is complete transposon.
- DS activity is depends on AC element.
- DS is incomplete transposon

DS - do not contain transposase although it contain TIR.

Transposase tnt in AC Element

Endosperm consist of millions of cell.

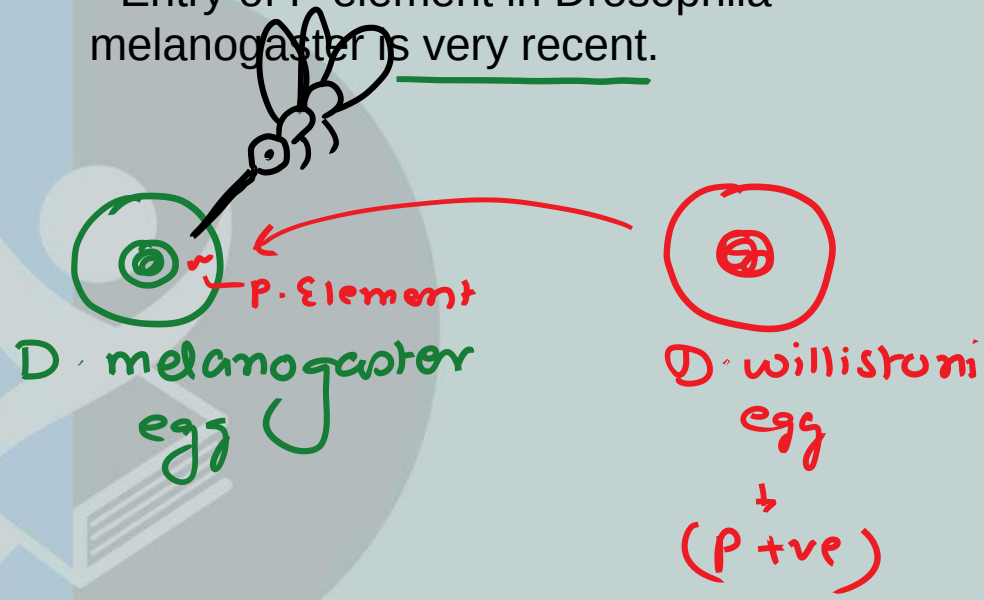


4. P elements of Drosophila.

- Contain TIR,
- transposase gene,
- target site duplication.
- Size ≈ 2900 base pair.
- Cause hybrid dysgenesis.

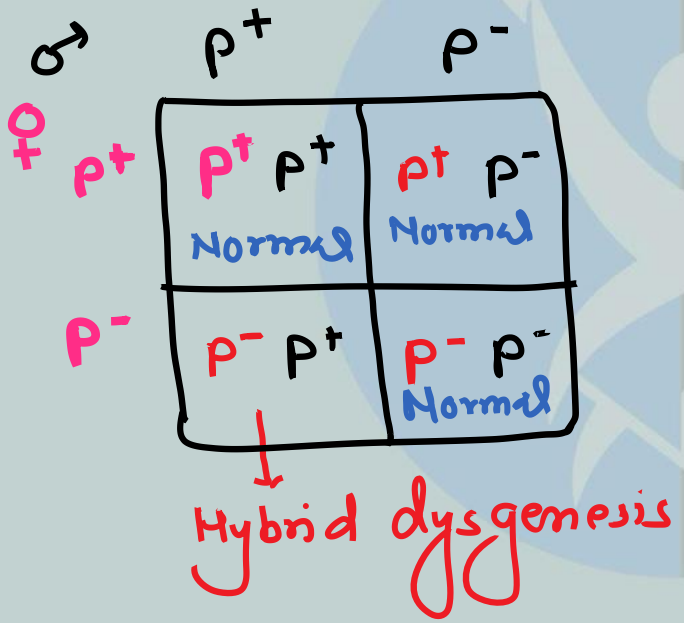
♀ P -ve × ♂ P +v → Hybrid dysgenesis

* Entry of P element in Drosophila melanogaster is very recent.



P positive cytotype = P element present.

P negative cytotype is = P element is absent



P. Element Can Be Regulated

1. If female is P positive

-> P element regulatory protein present in egg.

↓

P element can be regulated and prevent hybrid dysgenesis.

2. If female is P negative.

↓

P element regulatory protein absent.

If male is P positive then it fertilize

↓

P negative egg. Due to absence of regulatory protein,

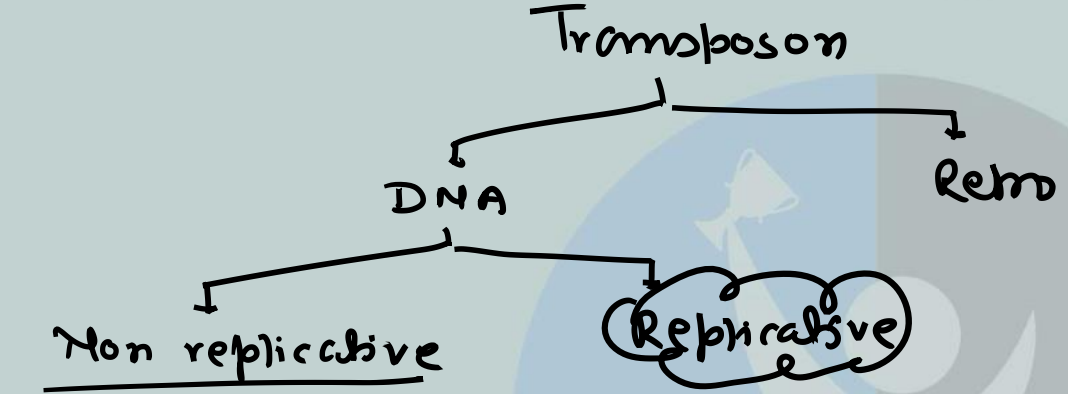
↓

P element can cause insertional inactvation of gene

↓

Hybrid Dysgenesis

Hybrid dysgenesis → When ♀ is P -ve
♂ is P +ve



-> Is Element
- Composit transposon
- AC DS Element
- P Element

#. Replicative transposon.

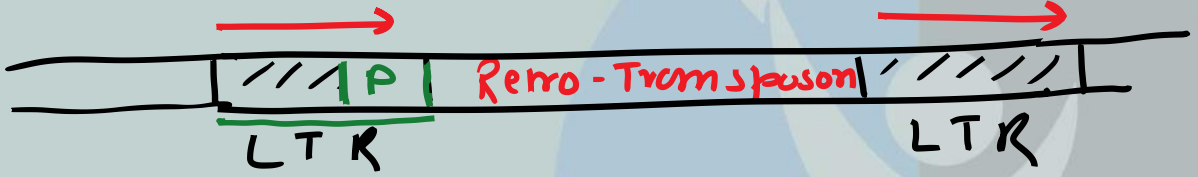
-> Copy paste type,
-> terminal inverted repeats present,
-> target site duplication,
-> transposase present,

Resolvase present.

Example, TN3.

2. Retro transposon.

- instead of TIR, long terminal repeats (LTR) present i.e. (direct repeats.)

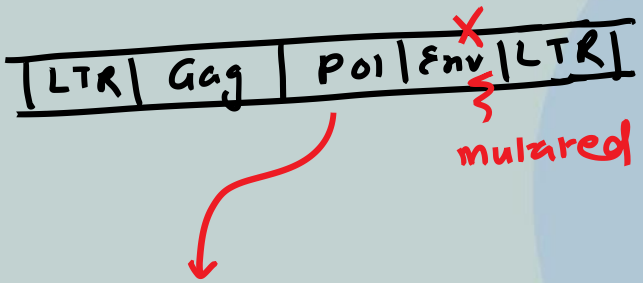


- Integrase present instead of transposase.
- Reverse transcriptase present,
- promoter present in LTR.

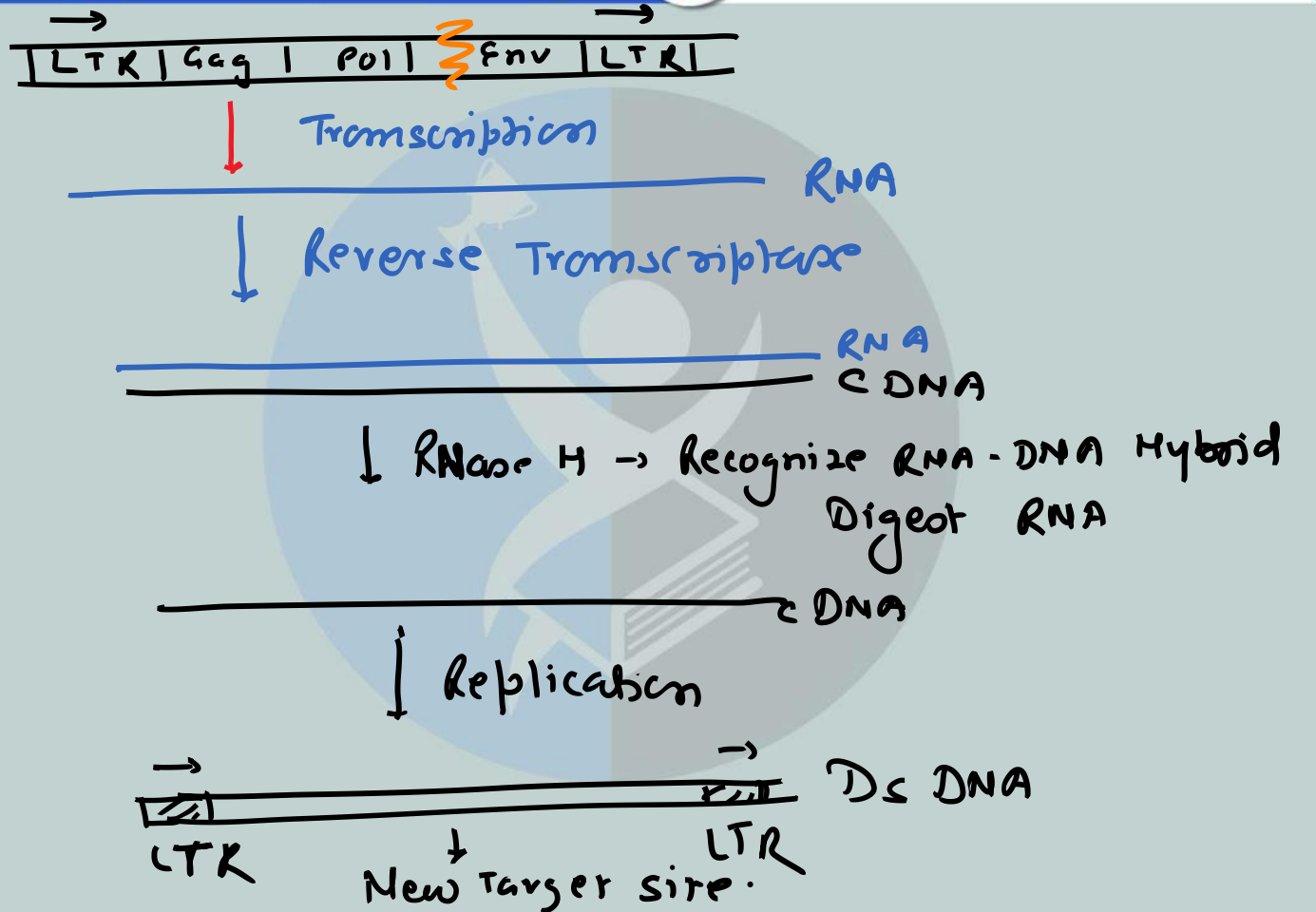
Retro transposon,

viral based

poly A transposon.



- RNAse H,
- reverse transcriptase,
- integrase.


LTR | Gag | Pol | Env | LTR
Transcription
RNA
Reverse Transcriptase
RNA
cDNA
RNase H → Recognize RNA-DNA Hybrid
Digest RNA
cDNA
Replication
Ds DNA
LTR
LTR
New Target site.

Eg. Ty transposon of yeast.

Gypsy, copia,

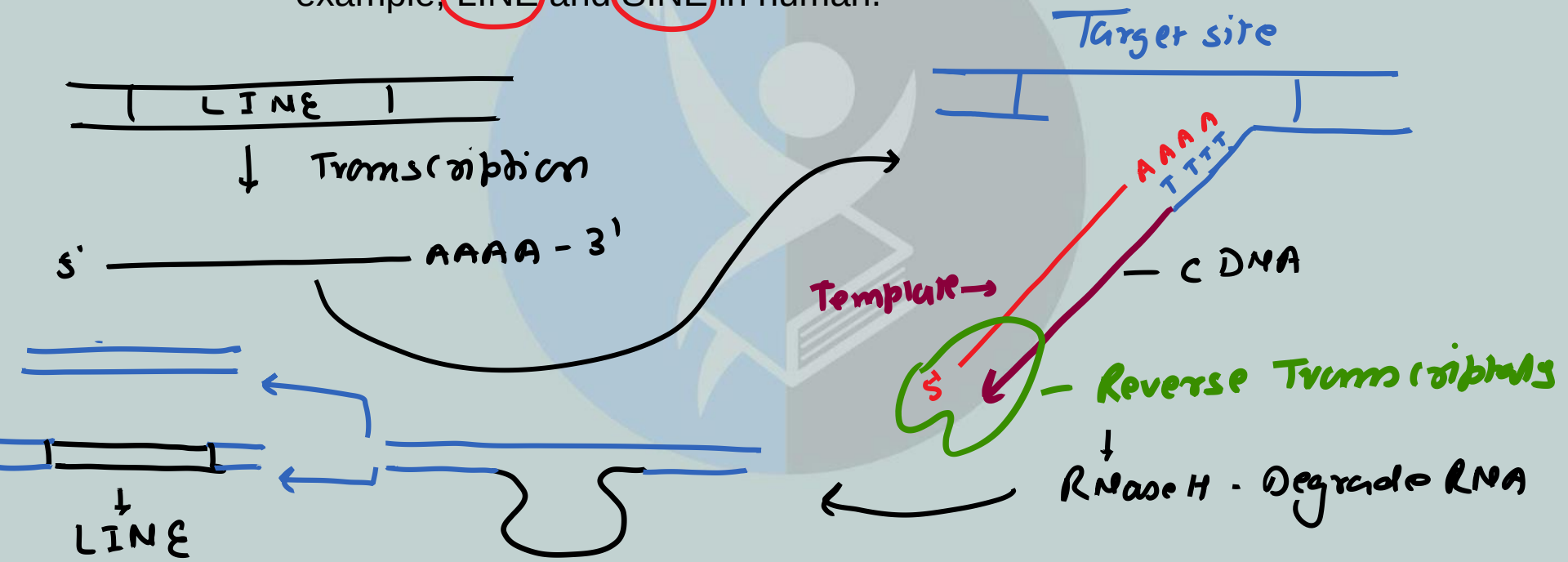
HET-A, TART present in drosophila and maintain telomeric length.

- **poly A transposon.**

Compleat Transposon

incompleat Transposon depends upon LINE

example; LINE and SINE in human.



Function of transposon.

1. Cellular function hypothesis-

- gene regulation,
- telomere length maintain.

2. Genetic variation hypothesis

Mutation ->variation -> evolution.

3. Selfish DNA hypothesis Accepted

- parasitic DNA
- invader DNA
- increase copy number
- do not take care of genome

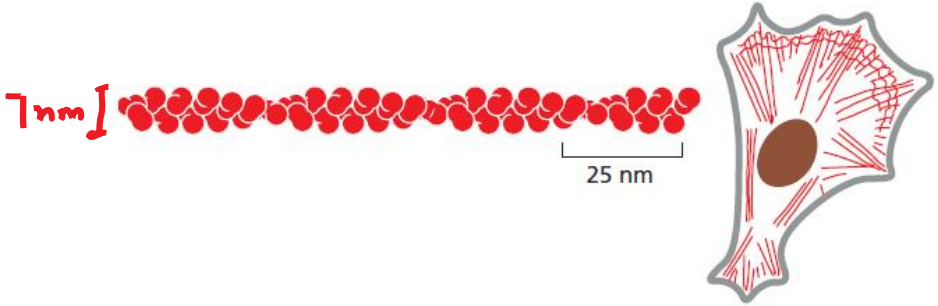
Application =>

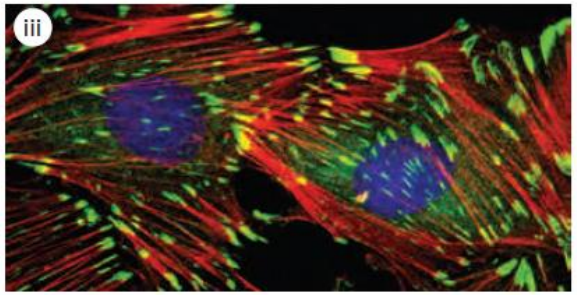
- random mutagenesis
- knockout experiment
- used as vector for DNA delivery.

Cytoskeletons

Cytoskeletons determines ➜ cell shape, cell movement, intracellular movement, vesicular transport, chromosomal movement.

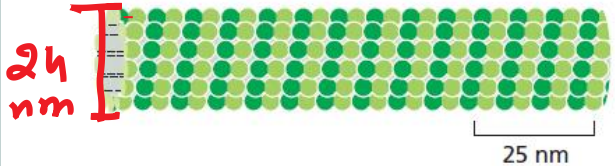
MICROFILAMENT [ACTIN]

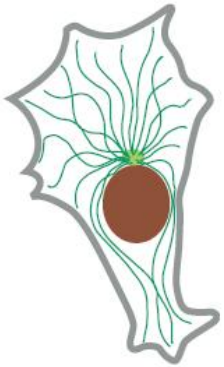


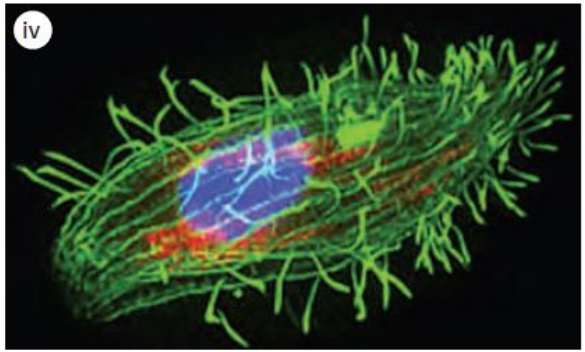


(Periphery)
→ maintain cell shape

MICROTUBULES



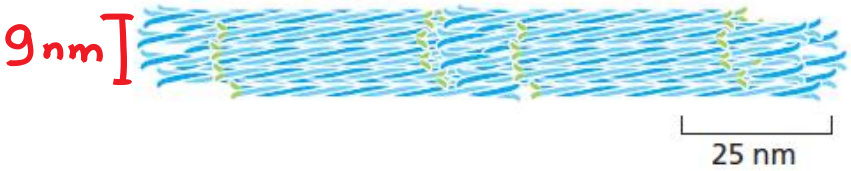


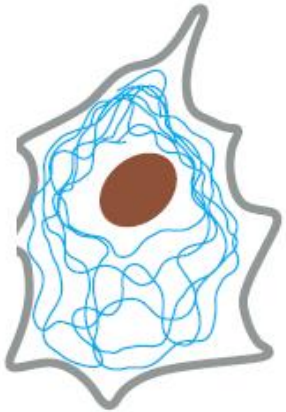


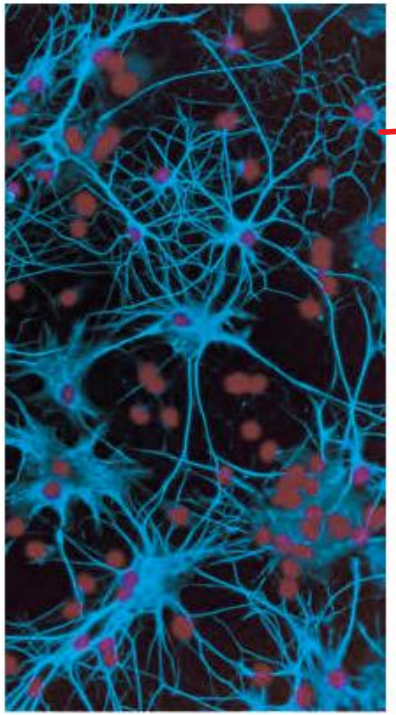
→ Center

→ Intracellular movement

INTERMEDIATE FILAMENTS





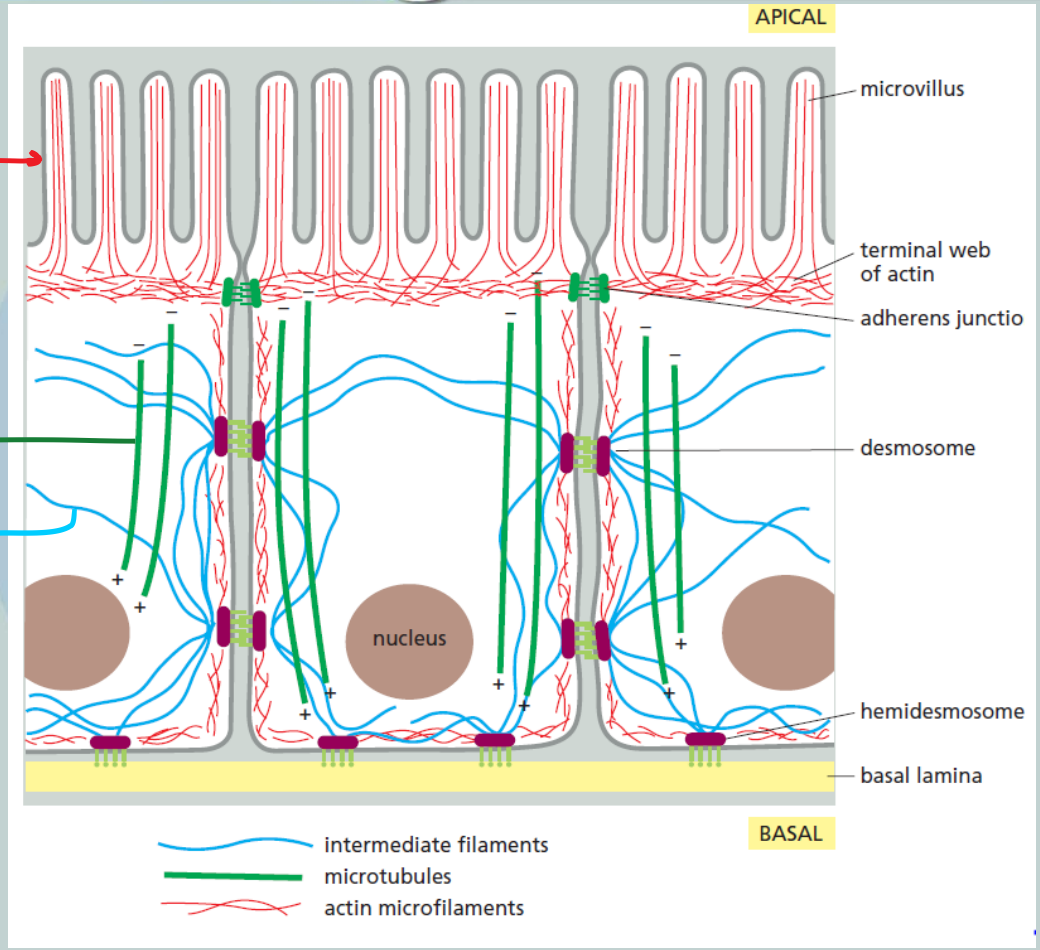


→ Provide Rigidity to The cell

microvill
[Small Projection
By microfilament]

microtubule

Intermediate
Filament.



Cytoskeleton

	Microtubule	Microfilament	Intermediate filament
Structure	Hollow tube with a wall consisting of 13 protofilaments	Two interwined chain of F actin consist of helically arranged G-actin	Eight protofilament joined end with staggered overlaps
Diameter	Outer: 25 nm, Inner: 15 nm	7nm	8-12 nm
Monomer Protein	α and β tubulin	G-actin	keratin, desmin, vimentin etc
Polarity	(+) and (-) ends	(+) and (-) ends	No polarity
Functions	Localization of cell organalles	Muscle contraction	Structural support
	Maintenance of animal cell shape and polarity	Cell locomotion	Maintenance of animal cell shape
	Chromosome movements	Cytokinesis	Formation of nuclear lamina and scalfolding
	Vesicular transport and movement of organelles	Movement of organelles at periphery of cell	Stengthening of nerve cell axons (neurofilament)
	Axonemal: Cell motility, flagellar movement	Present beneath membrabe, provide cell shape	Keeping muscle fibers in register (desmin)

microfilament, known as **Actin filament**,

1. Actin filament/ microfilament

responsible for –

- cellular movement, muscle contraction, cytokinesis, vesicular transport, filipodia, pseudopodia.

present toward periphery of the cell and maintain cell shape.

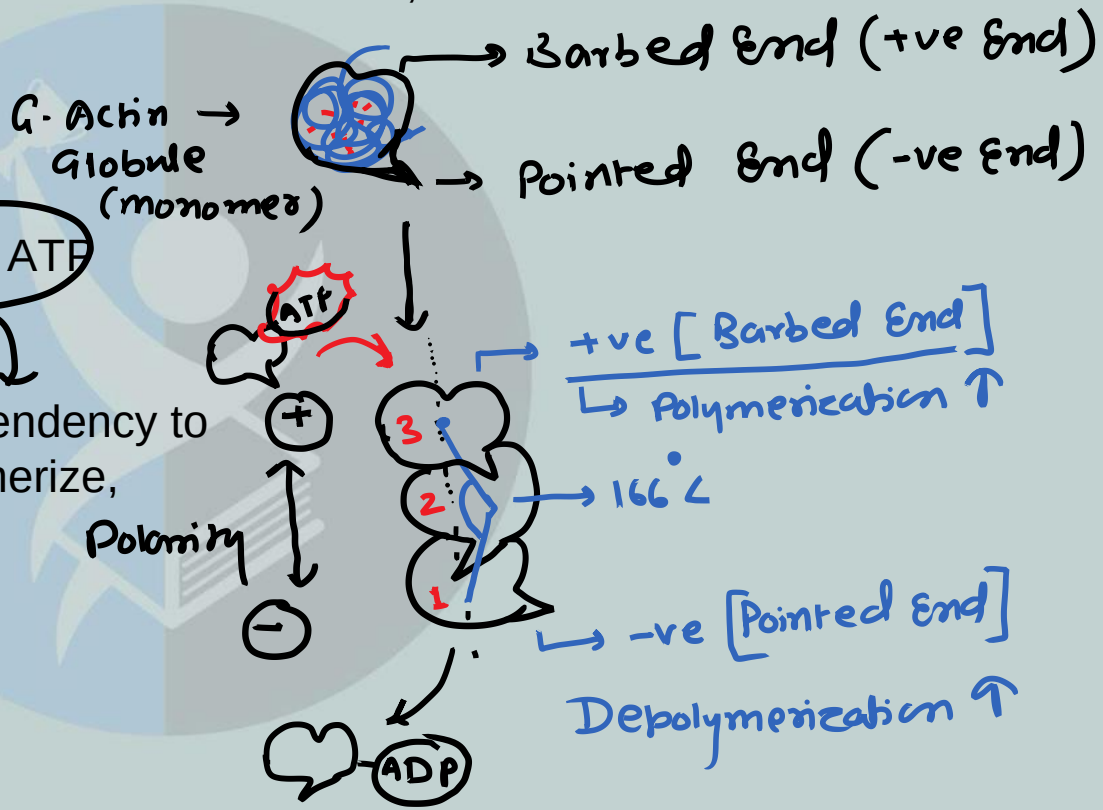
Structure and arrangement of actin filament,

actin is a globular protein, ✓
monomer, known as G-actin,

G-actin ATP, G-actin ADP G-actin ATP

tendency to depolymerize

has tendency to polymerize,

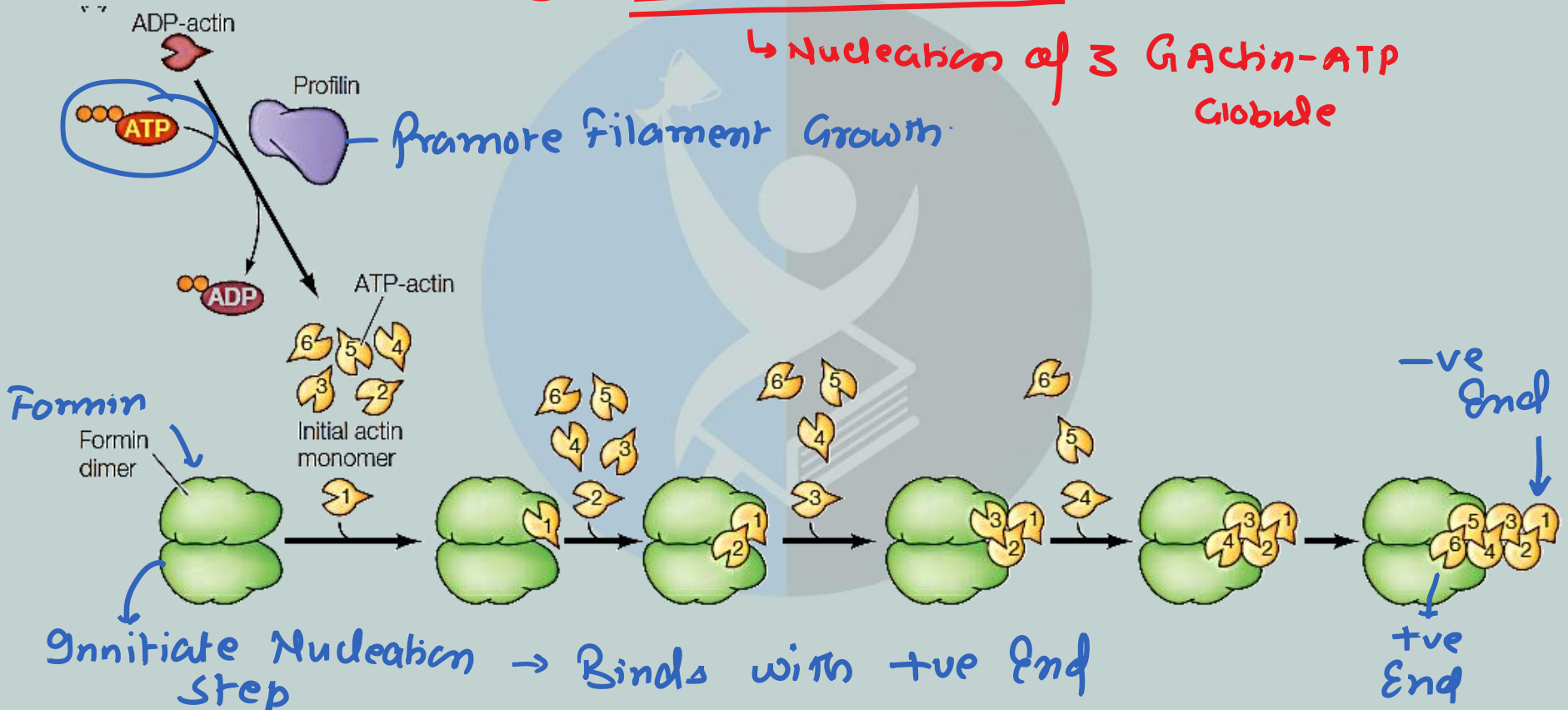


In vitro filament formation,

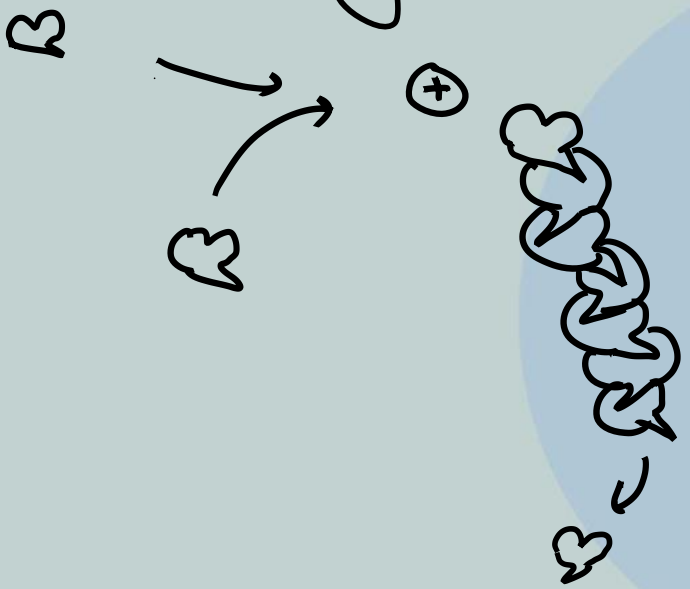
1. Nucleation- three actin globules polymerize first.
2. Elongation – filament length increase
3. Dynamic equilibrium- filament length is static, but filament is in dynamic state.

1 Nucleation Step

↳ Nucleation of 3 GActin-ATP Globule



Elongation = Polymerization > Depolymerization

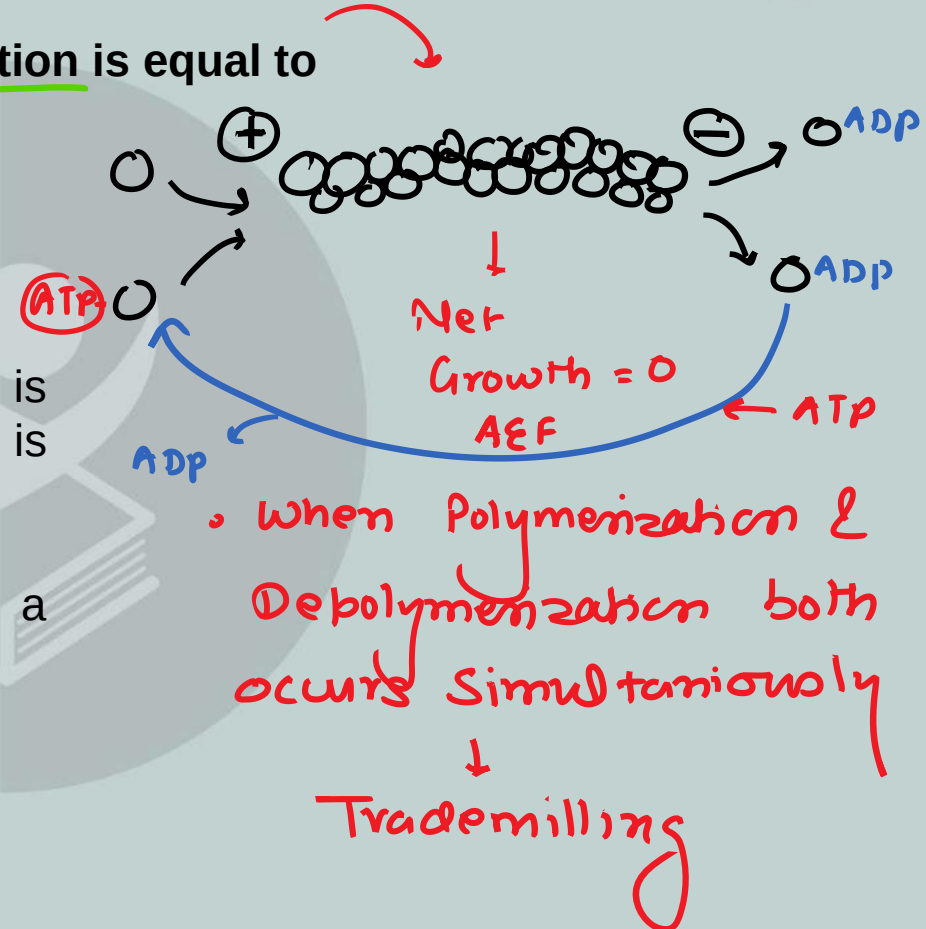


Dynamic equilibrium - Rate of polymerization is equal to rate of depolymerisation.

Net growth of filament is zero.

Trade milling is observed when one end is polymerizing and another end is depolymerizing.

Trade milling, recycling of actin globule in a filament.



if Polymerization Fast
Depolymerization Slow
→ trademilling Slow

if Both fast → trademilling fast
Both Slow → trademilling Slow

Actin - Polymerization and depolymerization is depends upon Critical conc of G Actin ATP

minimum conc of GActin - ATP That is required to start Polymerization

if GActin-ATP conc > Critical conc [Crc] = Polymerization ↑

if G Actin ATP conc < Crc = Polymerization ↓ Depolymerization ↑

CrC is different for +ve and -ve End

CrC for +ve End is lesser Than -ve En

eg CrC +ve End = 0.2 mM

CrC -ve End = 0.6 mM

if Conc. of G Actin in Sample: 0.3 mM

Filament Shrink ← [Depolymerization Rate (From -ve End) > Polymerization Rate (+ve End)

For Dynamic Equillibrium

Polymerization Rate = Depolymerization Rate

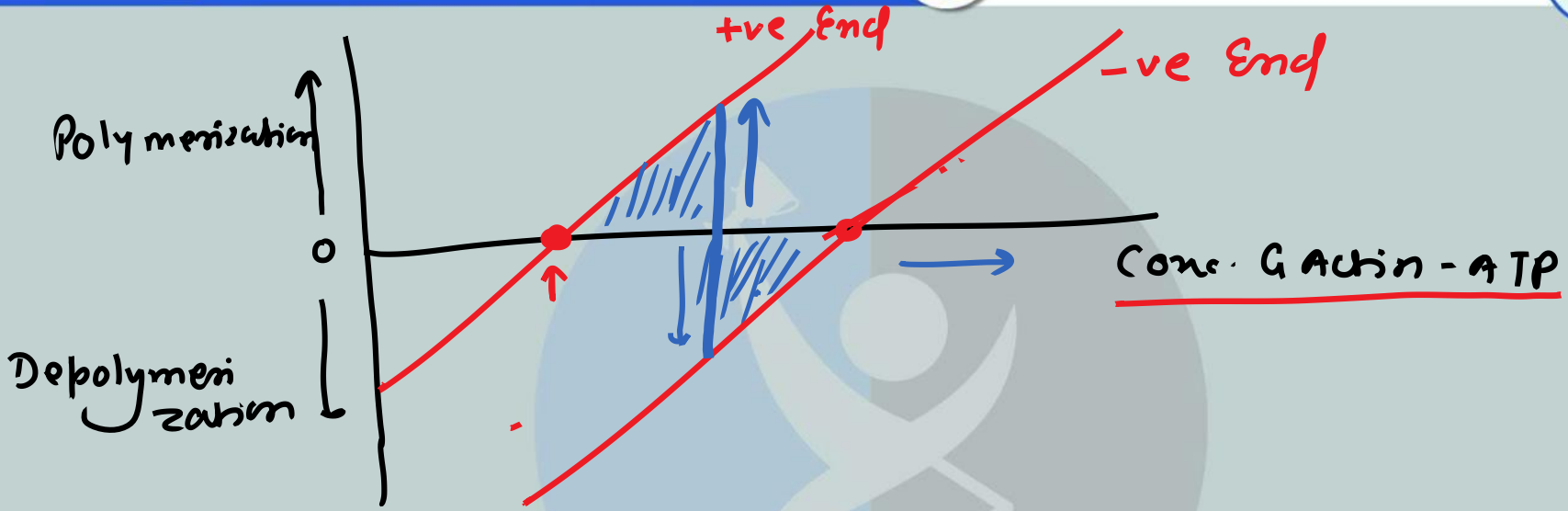
$$\frac{0.6 + 0.2}{2} = 0.4 \text{ mM}$$

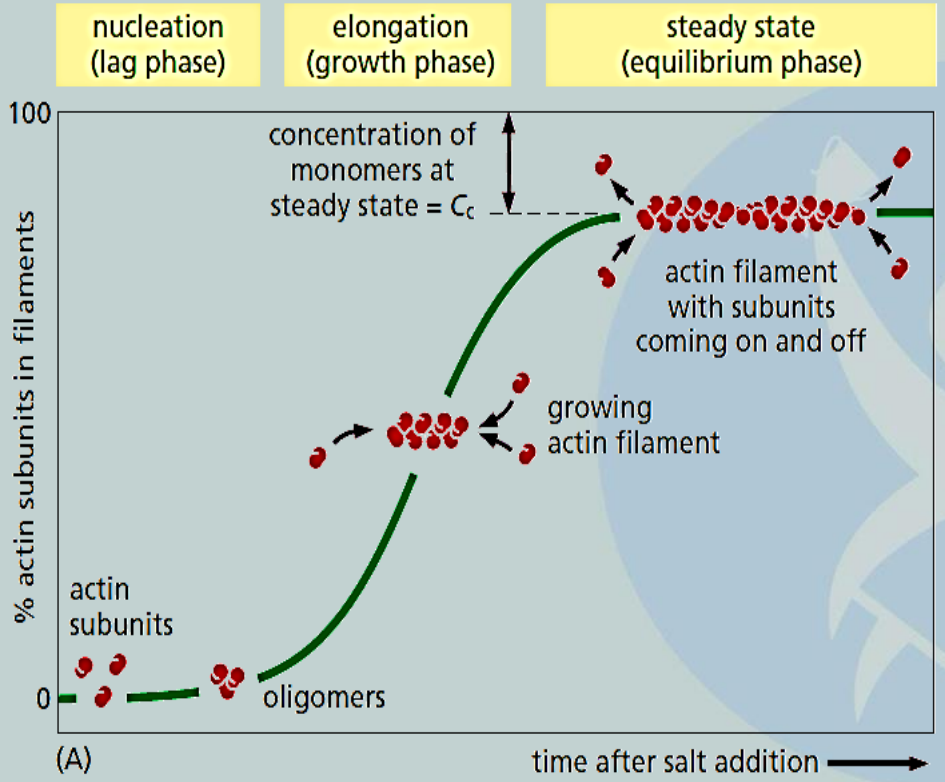
→ Conc. of GActin ATP

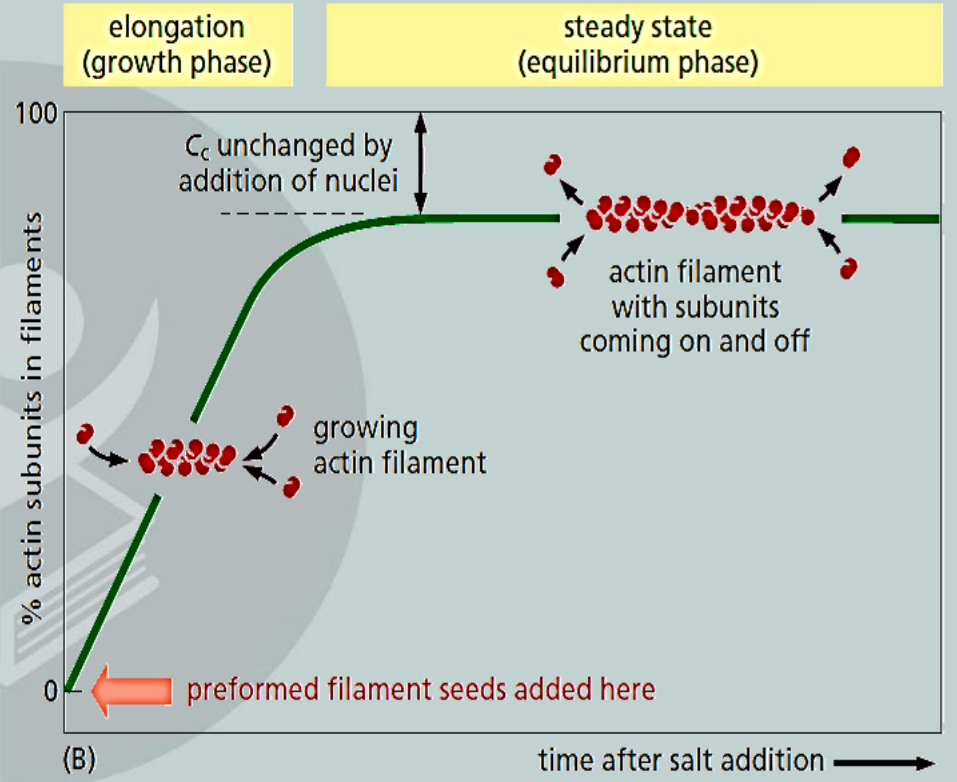
↓

At This Conc. of GActin ATP

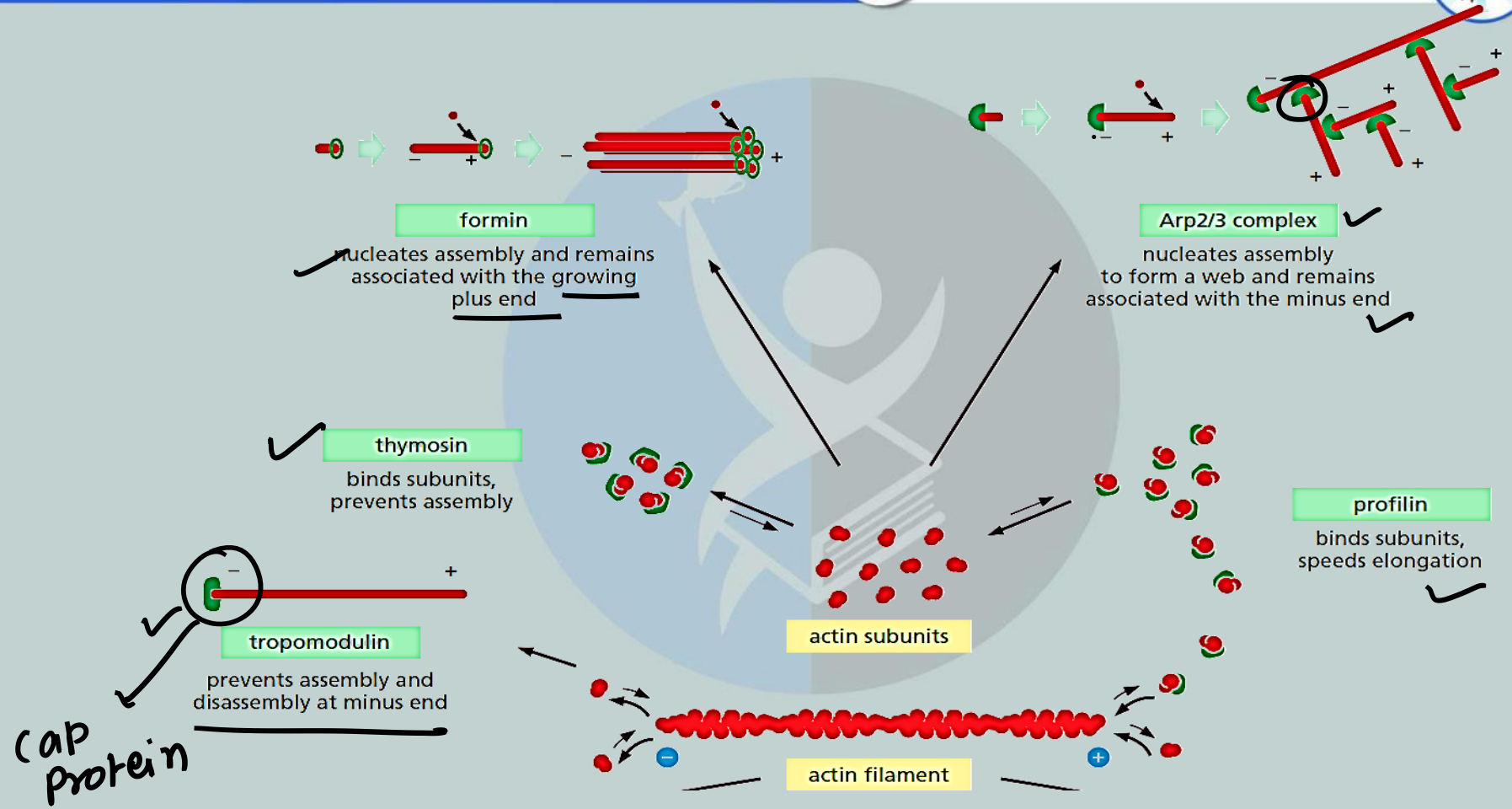
Dynamic Equillibrium can be seen.

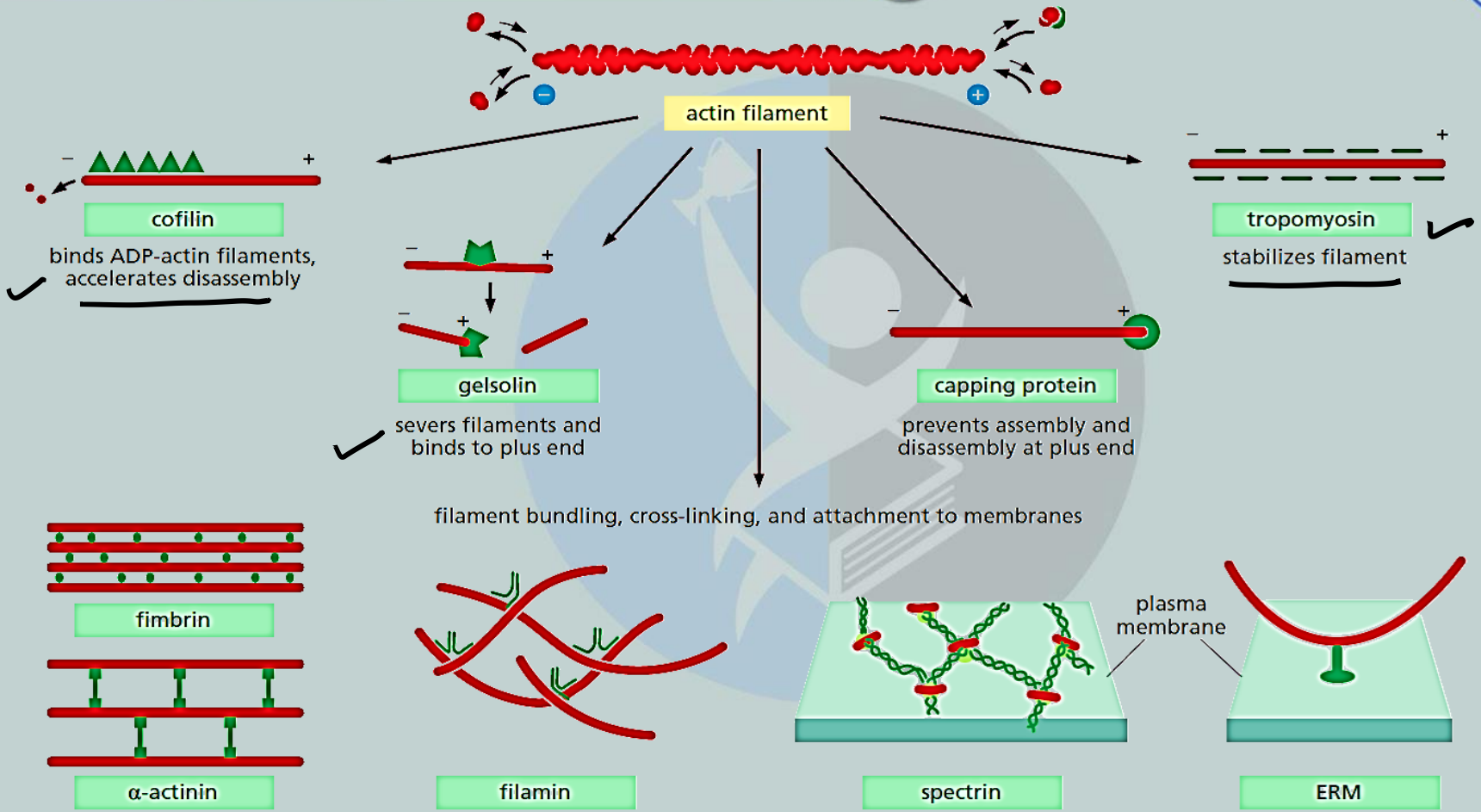

+ve End
-ve End
Polymerization
0
Depolymerization
Conc. G Actin - ATP


nucleation (lag phase)
elongation (growth phase)
steady state (equilibrium phase)
100
% actin subunits in filaments
0
concentration of monomers at steady state = C_c
actin filament with subunits coming on and off
growing actin filament
actin subunits
oligomers
(A)
time after salt addition


elongation (growth phase)
steady state (equilibrium phase)
100
% actin subunits in filaments
0
C_c unchanged by addition of nuclei
actin filament with subunits coming on and off
growing actin filament
preformed filament seeds added here
(B)
time after salt addition

Actin - interacting Protein


formin
nucleates assembly and remains associated with the growing plus end
Arp2/3 complex
nucleates assembly to form a web and remains associated with the minus end
thymosin
binds subunits, prevents assembly
profilin
binds subunits, speeds elongation
tropomodulin
prevents assembly and disassembly at minus end
cap protein
actin subunits
actin filament


actin filament
cofilin
binds ADP-actin filaments, accelerates disassembly
gelsolin
severs filaments and binds to plus end
capping protein
prevents assembly and disassembly at plus end
tropomyosin
stabilizes filament
filament bundling, cross-linking, and attachment to membranes
fimbrin
α-actinin
filamin
spectrin
plasma membrane
ERM

Cellular Role	Representative proteins
Promote Monomer binding	Profilin, twinfilin
Filament initiation and polymerization	Arp2/3, formin
End capping	CapZ, tropomodulin → +ve End cap → −ve End cap
Filament stabilization	Nebulin, tropomyosin
Filament cross-linking	α-actinin, filamin, fimbrin, villin
Actin filament linkage to other proteins	Dystrophin, spectrin, talin, vinculin
Filament severing	Cofilin

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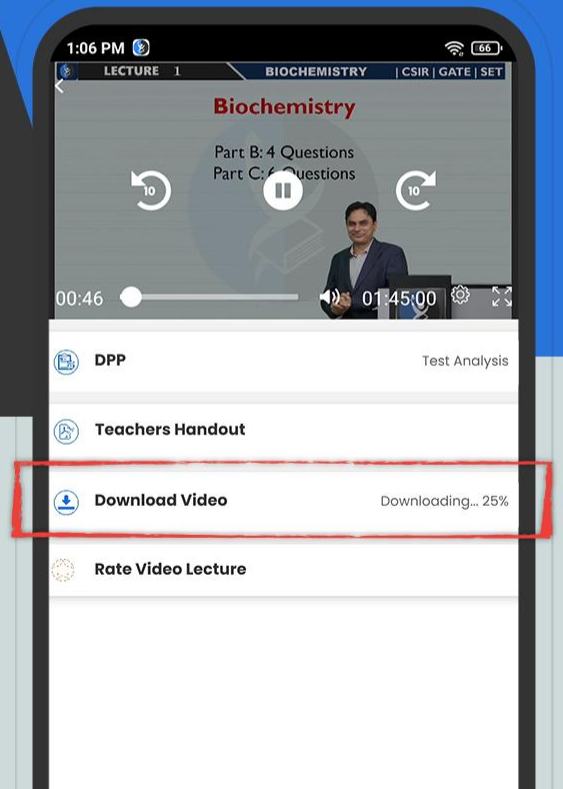
Minimize Internet Usage by Downloading Lectures from the IFAS App & Access them Offline



IfAS

Harshada Sharma

Earned: ₹25,500

"As is widely recognized, IFAS stands as India's leading institute for CSIR NET examination preparation. My Personal philosophy has always revolved around helping others and I've had the pleasure of introducing the IFAS Online Course to 12 of my classmates and juniors, which in turn allowed me to earn 25,500 rupees. You too can join in and refer your friends, allowing you to earn a 5% rewards based on the fees each of your referred friends Pay"

REFER & EARN

Step – 01 Invite Friends

Share your referral code or Link with your friends.

Step – 02 Earn Cashback

On Every Purchase made using your referral code, you earn 5% instant cashback in your bank account.

Step – 03 Refer More, Earn More

Keep referring as many friends as you can and keep **Earning** cash as much as you can

Friendship is Sharing, Caring, Giving & Helping Each Other...!